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# Use of steinernematid nematodes for post harvest control of navel orangeworm (Lepidoptera: Pyralidae, *Amyelois transitella*) in fallen pistachios

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**Abstract**

Four trials employing 1-$m^2$ plots were conducted between November 2002 and April 2003 in Madera County, California, to evaluate the ability of two species of nematodes, *Steinernema carpocapsae* and *Steinernema feltiae*, to control navel orangeworm, *Amyelois transitella*, in infested pistachios on the ground. The plots were located in two 16.2 ha blocks of pistachio trees planted in sandy loam soil. A total of 4300 larvae were recovered from 17,593 laboratory-infested pistachios (24.4% average infestation). Nematodes were applied with a backpack sprayer at concentrations ranging from 50,000–1,000,000 infective juveniles (IJs)/$m^2$ (100,000 IJs/$m^2$ was assessed in all four trials) and an application rate of 374 or 500 ml/$m^2$ water. *S. carpocapsae* was more effective than *S. feltiae* in pistachios and produced >72% mortality at a concentration of 100,000 IJs/$m^2$ when nighttime temperatures were above freezing. *S. carpocapsae* was equally effective in bare and leaf-covered plots and persisted longer in sandier soil (8 weeks) than *S. feltiae*. *S. carpocapsae* has the potential to multiply in the field; 51.3% of the cadavers examined 21 days after application contained developing nematodes ($n = 226$). Our trials demonstrated that *S. carpocapsae* can play a role in the post harvest control of navel orangeworm and that the formulation tested produced greater mortality than the formulations of *S. feltiae* tested at the same concentration.
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*Keywords:* *Steinernema carpocapsae*; *Steinernema feltiae*; *Amyelois transitella*; Mortality; Pistachios; Almonds; Biological control; Entomopathogenic nematodes; Post harvest

## 1. Introduction

The navel orangeworm (NOW), *Amyelois transitella* (Walker), is a key pest of pistachios in California. The pistachio nut is vulnerable to infestation when the hull splits and NOW is controlled during the growing season by the application of organophosphate, carbamate, and other insecticides (Bentley et al., 2000; Zalom et al., 2002). NOW larvae overwinter in fallen nuts and nuts left on trees and continue to develop inside the nut throughout the winter as temperature permits. Initial invasion of pistachio orchards occurs when the overwintering generation emerges in early March through May. Overwintering larvae are unaffected by insecticides and are controlled by field sanitation. Sanitation is accomplished by removal of the nuts from the trees and subsequently tilling fallen pistachios into the soil. Mortality of NOW in intact nuts is dependent on the depth that the nuts are buried (B. Higbee, unpublished) and whether the nuts decompose over the winter.

Entomopathogenic nematodes (EPNs) are effective in controlling a wide variety of insects in soil and cryptic habitats including those found in orchards (Klein, 1990; Lacey et al., 2000). The cryptic overwintering site of NOW in fallen pistachios, especially in combination with shallow tillage, provides an opportunity for its

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control using EPNs. Initial evaluations of *Steinernema carpocapsae* (Weiser), conducted against NOW in almonds, demonstrated activity against larvae in nuts on trees during the growing season (Agudelo-Silva et al., 1987; Lindegren et al., 1987). A subsequent study by Agudelo-Silva et al. (1995) evaluated the efficacy of steinernematid and heterorhabditid nematodes against NOW in almonds left on trees after harvest and found them ineffective (11.8% mortality); the study did not evaluate fallen nuts. The susceptibility of NOW larvae in pistachio nuts to EPNs has not been investigated. The purpose of our study was to evaluate the ability of two commercially available steinernematid species, *S. carpocapsae* and *Steinernema feltiae* (Filipjev), to infect NOW larvae in pistachios on the ground, in order to determine if EPNs can play a role in pistachio orchard sanitation. We report the results of four small plot field trials conducted from November 2002 through April 2003, and one large plot field trial conducted in November 2002.

## 2. Materials and methods

### 2.1. Study site description and small plot experimental design

The studies were conducted in two 16.2 ha blocks (Block 1: AGL 72 12-11; Block 2: AGL 72 12-14) located in a pistachio orchard operated by S & J Ranch (Madera, CA). The trees were planted in sandy loam soil. Moisture levels varied between the two blocks and Block 2 soil had a lower percent relative saturation (higher sand content). This was determined in April 2003 by selecting two spots at random within each row and inserting a Kelway HB-2 Soil Tester (Kel Instruments, Wyckoff, NJ) for a total of 10 measurements per block. Percent relative saturation is a measure of a soil type's ability to hold water and is 100% when the soil type is at its field capacity (field capacity for fine sand $\approx$ 32%, loam $\approx$ 45%, clay $\approx$ 65%).

Small plots (1-$m^2$) were placed on the north side of the berm in front of microsprinklers. The plots began at the third tree from the road to minimize edge effects and were placed consecutively unless a tree was missing or a sapling was present. When this occurred, the next mature tree down the row was used to continue the plots. The plots were marked using colored flags on 35 cm wire spikes. The distribution of the treatments among the plots within a row was completely randomized. One day before application, the plots were irrigated for 2 h by the micro-sprinklers (22.7 liters/h).

Trial 1, November 6–26, 2002, consisted of 50 bare plots (pistachio leaves had not yet fallen) in Block 1 and each row contained two replicates of every treatment. Each plot contained two sets of infested pistachios (30 nuts/set) and two sets of infested almonds (30–40 nuts/set). One almond set was left on the surface and the remaining sets were partially buried by hand sprinkling soil on top of them. All sets were placed on soil-covered netting squares (625 $cm^2$) on the day of application in order to facilitate easy removal of infested nuts and larvae. These netting squares were used for all trials. The plots were covered with nylon mesh (held in place with nails) 48 h after application. One set of pistachios was randomly selected and removed 7 days after treatment and the remaining set was collected 21 days after application. All almonds were collected 7 days after application. All collected nuts were placed in paper bags marked by row and treatment, stored in plastic tubs, brought back to the laboratory within 3 h after collection and held at 10 °C until the larvae were assessed. This procedure was followed for all trials.

Trial 2, February 28–March 9, 2003, consisted of 60 plots in Block 1 and each row contained a single replicate of each treatment. There were two sets of nuts per plot (one set of infested pistachios, 45 nuts/set and one set of infested almonds, 25 nuts/set) and all nuts were partially buried. Half of the plots in this trial were covered with a single layer of pistachio leaves. Immediately after nematode application and wetting, the plots were covered with nylon mesh. The nuts in half of the plots were randomly selected and collected on day 7 and the remainder collected 10 days after application.

Trial 3, March 20–26, 2003, consisted of 60 plots in Block 2 and each row contained a single replicate of each treatment. Only partially buried pistachios were used (80 nuts/set). Half of the plots were covered with a single layer of pistachio leaves and none of the plots were covered with nylon mesh. The pistachios were collected 7 days after application.

Trial 4, April 23–29, 2003, consisted of 60 plots evenly divided between Blocks 1 and 2. Only partially buried pistachios were used (120 nuts/set). All plots were covered with a single layer of pistachio leaves and then covered with nylon mesh 24 h after application. The nuts were collected 7 days after application.

### 2.2. Evaluation of nematode efficacy and persistence in large-scale application

Four adjacent rows were selected (0.2 ha area/row) and the area between the rows randomly assigned as a Control, *S. carpocapsae*, or *S. feltiae* plot. Each plot was tilled with a disk harrow (depth approximately 10 cm) 1 day before application. Within each plot, 7 1-$m^2$ sites were selected at random and marked with flags. Each site received two sets of almonds (approximately 20–30 nuts per set), one of which was half buried and the other set was placed on the surface. Immediately before nematode application, the large plots were irrigated using a watering truck at 1870 liters/ha (187 ml/1-$m^2$

site). Two plots received either *S. carpocapsae* or *S. feltiae* (50 IJs/cm$^2$) at an application rate of 1870 liters/ha water followed by 1870 liters/ha water. The remaining plot served as a control and received water at the same rate. The nematodes were applied using two flat fan nozzles mounted on a tractor-pulled herbicide sprayer. These sets were not covered with screen after application and the almonds were collected 7 days after application. Soil samples (approximately 2 kg) from these large plots were collected 6 and 12 weeks after application to evaluate nematode persistence.

### 2.3. Source of nematodes and preparation

Trial 1 utilized laboratory-produced *S. carpocapsae* in the small plot experiment. The nematodes were grown in greater wax moths (*Galleria mellonella* (L.)) and collected according to Kaya and Stock (1997). The large plot trial and the subsequent small plot trials used liquid commercial formulations of *S. carpocapsae* produced by Certis USA (Columbia, MD) in a bioreactor located at Wasco, California. These nematodes were transported to our laboratory in continuously aerated containers (aquarium pump and air stone) and stored according to the producer's instructions at 10 °C until use. The same lot of *S. carpocapsae* was used for the February, March, and April small plot experiments. All of the *S. feltiae* used was produced commercially. Becker Underwood (Ames, IA) produced the nematodes used in the November trial and BioLogic (Willow Hill, PA) produced the nematodes used in February and March. The Becker Underwood nematodes, received as a gel formulation produced in Littlehampton, United Kingdom, were shipped with an icepack and received 1 day before use. These nematodes were immediately mixed with bottled distilled water (Arrowhead, Brea, CA) upon receipt and stored in a continuously aerated container overnight at 10 °C. *S. feltiae* from BioLogic was shipped with an icepack and received 1 week before use. These nematodes were formulated on sponge and were stored immediately upon receipt in a refrigerator at 4 °C according to the producer's instructions. One half of the sponge was used in the February trial and the other half used in the March trial. For all small plot trials, the nematode preparations were mixed with bottled distilled water, counted 1 day before application, and the final concentrations prepared. The final concentrations were stored overnight at 10 °C in continuously aerated containers and checked the following morning to ensure viability. The nematodes were then transported to the field in continuously aerated containers. The containers were kept in shade in the field (no more than 2 h) until application. In the large plot trial, nematodes in continuously aerated containers were poured directly into the tank of the herbicide sprayer and water was added to the tank to make the final concentration.

### 2.4. Infestation of nuts

Trials 1 and 2 utilized unprocessed pistachio nuts (possessed hulls, moisture content 18–20%). Unprocessed nuts are similar in moisture content to pistachios in the field but deteriorate rapidly in storage because the hull becomes moldy. When the supply became exhausted, partially processed nuts (hulled, partially dehydrated) received from a Paramount Farming Company (Bakersfield, CA) storage facility were used in the remaining trials. Hulled nuts can be held for long periods and support the development of NOW larvae. Nuts that have shed their hulls are found in the field and are naturally infested with NOW. A laboratory colony of NOW was used to infest all of the pistachios with second–fourth instar larvae. The nuts were incubated at 22–24 °C for 3–7 days in order to allow the larvae to establish and then stored at 10 °C to arrest larval development until use (a maximum of 2 weeks). Infested nuts were removed from the incubator and held at room temperature (22–24 °C) for 2 days before placement in the field. The range of NOW instars present in these infested nuts was similar to the range present in the field. The average pistachio infestation was 24.4% in the four trials and the majority of the infested nuts had a single larva. Naturally infested Nonpareil almonds (50% NOW infestation, 300 nuts examined) were received from Paramount Farming Company and used in the first and second trials.

### 2.5. Application rates used for the small plots

Nematodes were applied using a $CO_2$ pressurized sprayer calibrated at 207 kPa (30 psi) and a hand-held, two nozzle spray boom equipped with TeeJet TP8010 nozzles (Spraying Systems, Wheaton, IL). The tip strainers were removed to reduce shearing of the nematodes. The nematodes were sprayed directly over the exposed nuts or pistachio leaves at a height of 40–60 cm. In Trial 1, 10 plots received 100 IJs/cm$^2$ and 10 plots received 10 IJs/cm$^2$ of laboratory-produced *S. carpocapsae* or commercially produced *S. feltiae*. The nematodes were applied in 374 ml of water/plot followed by 500 ml of water to facilitate nematode dispersal into nuts and soil. In Trials 2 and 3, all plots received a concentration of 10 IJs/cm$^2$. Half of the plots received 374 ml of water after application and the remainder received 500 ml water. In Trial 4, half of the plots received a concentration of 10 IJs/cm$^2$ and the remainder received a concentration of 5 IJs/cm$^2$. All plots received 374 ml of water after application. The control plots received 374 ml water followed by either 374 or 500 ml water (depending on treatment) delivered by the pressurized sprayer. All plots were irrigated by micro-sprinklers for 2 h (22.7 liters/h) within 6 h after application. The plots received additional irrigation in subsequent days from

microsprinklers and/or rainfall. In Trial 4, soil samples were collected from the plots 4 and 8 weeks after application (5 plots sampled per block, approximately 2 kg per treatment) to evaluate persistence.

### 2.6. Determination of mortality, nematode multiplication, and nematode persistence

All split pistachios were opened and examined for larvae; nuts that did not split (10%) were not included in the analysis because NOW could not infest them. All almonds were examined for larvae and used in the analysis. In this study, larvae were considered alive if they moved when prodded, and pupae or the rarely recovered pupal case were scored as live larvae. A subset of the dead larvae were examined with a compound microscope at 60× magnification to confirm infection for each trial; when adults and early stage nematodes were observed in the cadavers, we considered multiplication to have occurred.

We evaluated persistence by mixing soil samples from each treatment in sterilized buckets and then selecting 900 g of soil per treatment. The soil was evenly divided into 3 containers per treatment, moistened with distilled water, and 10 greater wax moth larvae added per container according to Kaya and Stock (1997). Persistence of *S. carpocapsae* and *S. feltiae* was evaluated in the large plot experiment in Trial 1 and persistence of *S. carpocapsae* in Blocks 1 and 2 was evaluated for Trial 4. Mortality was assessed at 7 days and we considered persistence established when mortality was significantly greater in the nematode-treated samples than the control samples. Cadavers with emerging nematodes were placed in new containers containing larvae to confirm that the nematodes caused mortality.

### 2.7. Temperature recording

Two HOBO data loggers (Onset Computer Corporation, Bourne, MA) were used to monitor soil temperature at a depth of 2–4 cm and two HOBO data loggers monitored air temperature at approximately 1.5 m above ground (placed on the underside of a branch). Monitoring began 1 day before application and ended on the day when the nuts were collected. The average of the two soil temperature probes was calculated and reported in this paper. Air temperature data were also collected from a weather station operated by S & J Ranch (located 1 km from the study site) and used to validate our HOBO data. In Trials 2, 3, and 4 the soil temperature probes were located under the pistachio leaves.

### 2.8. Statistical analysis

Multiple regression/correlation (mrc) analysis with dummy coding was used to evaluate mortality data and mrc analysis with orthogonal contrasts was used to compare differences among single and grouped treatment means (Cohen and Cohen, 1983). Two-way ANOVA and Fisher's protected LSD post hoc test were also used to analyze mortality data. Relative Risk (R.R.), a statistic used in epidemiology to evaluate the likelihood of a specified dichotomous outcome, was employed to evaluate differences in total larval recovery between EPN treatments and nematode persistence (Kelsey et al., 1986). An unpaired Student's $t$ test was used to evaluate differences in relative percent saturation between Block 1 and Block 2. In the mrc and two-way ANOVA analyses, data from control plots were pooled when there was no difference between sample dates or block, and treatment data were pooled similarly. In Trial 1, data were pooled when there was no difference between surface and half-buried almonds.

## 3. Results

### 3.1. Larval mortality in pistachios

Application of both nematode species significantly increased larval mortality ($P < 0.0001$, $t = -29.3, -5.2, -22.2, 35.8$, respectively) in all trials. Mortality did not differ over time in Trials 1 and 2 for either nematode species, and in Trial 4 control mortality was similar in both blocks (Table 1). Treatment mortality was lowest in Trial 2 although it still was greater than control mortality. However, there were differences in the mortality caused by the two species. *S. carpocapsae* caused greater mortality than did *S. feltiae* applied at the same concentration in Trials 1 and 3. In Trials 2 and 3 the efficacy of *S. carpocapsae* was unaffected by leaves covering the nuts. The mortality produced by a concentration of 10 IJs/cm$^2$ of *S. carpocapsae* was comparable in Trials 1, 3, and 4 but when the concentration was lowered in Trial 4, mortality decreased in Block 2. Overall, the efficacy of *S. feltiae* was more variable than *S. carpocapsae* in Trials 1–3. In Trial 3, *S. feltiae* produced greater mortality in bare plots than leaf covered plots that received 500 ml water after application ($P = 0.008$, $t = 2.65$) while the reverse occurred when 380 ml water was sprayed after application ($P = 0.019$, $t = -2.36$).

### 3.2. Larval mortality in almonds

Application of nematodes significantly increased larval mortality compared to the controls in the Trials 1 and 2 small plots ($P < 0.0001$, $t = -15.5, -7.3$, respectively) as well as the Trial 1 large plot ($P < 0.0001$, $t = -17.8$) (Tables 2 and 3). In Trial 1, both species of nematode were more effective in small than large plots ($P < 0.0001$, $t = -4.5$) and the two nematode species

Table 1
Mean mortality of navel orangeworm larvae in pistachios after treatment with *S. carpocapsae* and *S. feltiae*

| Trial | Species | IJs per cm$^2$ | Percent Mortality (±SE)[a] | *N* |
|---|---|---|---|---|
| November 2002, Block 1 | | | | |
| Bare plot | Control | | 16.8 ± 2.1 a | 322 |
| Bare plot, 500 ml | *S. carpocapsae* | 100 | 94.3 ± 3.2 b | 174 |
| Bare plot, 500 ml | *S. carpocapsae* | 10 | 89.7 ± 3.2 b | 280 |
| Bare plot, 500 ml | *S. feltiae* | 100 | 91.0 ± 2.8 b | 177 |
| Bare plot, 500 ml | *S. feltiae* | 10 | 74.0 ± 2.9 c | 238 |
| February 2003, Block 1 | | | | |
| Bare + Leaves plots | Control | | 19.0 ± 2.3 a | 384 |
| Bare plot, 374 ml | *S. carpocapsae* | 10 | 31.2 ± 3.8 b | 218 |
| Leaves plot, 374 ml | *S. carpocapsae* | 10 | 36.7 ± 3.8 b | 218 |
| Bare plot, 374 ml | *S. feltiae* | 10 | 36.9 ± 4.1 b | 176 |
| Leaves plot, 374 ml | *S. feltiae* | 10 | 28.6 ± 8.1 b | 217 |
| March 2003, Block 2 | | | | |
| Bare + Leaves combined | Control | | 8.9 ± 2.3 a | 292 |
| Bare plot, 500 ml | *S. carpocapsae* | 10 | 86.3 ± 6.0 b | 51 |
| Leaves plot, 500 ml | *S. carpocapsae* | 10 | 79.8 ± 5.4 b | 54 |
| Bare plot, 374 ml | *S. carpocapsae* | 10 | 72.2 ± 7.0 b | 36 |
| Leaves plot, 374 ml | *S. carpocapsae* | 10 | 78.5 ± 2.3 b | 65 |
| Bare plot, 500 ml | *S. feltiae* | 10 | 59.2 ± 5.2 c | 71 |
| Leaves plot, 500 ml | *S. feltiae* | 10 | 40.0 ± 6.1 d | 51 |
| Bare plot, 374 ml | *S. feltiae* | 10 | 34.8 ± 6.5 d | 43 |
| Leaves plot, 374 ml | *S. feltiae* | 10 | 55.4 ± 5.4 c | 65 |
| April 2003, Blocks 1 + 2 | | | | |
| Block 1 + 2 combined | Control | | 15.9 ± 2.6 a | 452 |
| Block 1 Leaves, 374 ml | *S. carpocapsae* | 10 | 80.6 ± 4.9 b | 67 |
| Block 2 Leaves, 374 ml | *S. carpocapsae* | 10 | 78.0 ± 3.2 b | 164 |
| Block 1 Leaves, 374 ml | *S. carpocapsae* | 5 | 68.2 ± 3.8 b | 151 |
| Block 2 Leaves, 374 ml | *S. carpocapsae* | 5 | 56.1 ± 3.8 c | 173 |

Infective juveniles (IJs) were applied to bare ground and leaf-covered plots. Application was followed by 374 or 500 ml water. Data from control plots were pooled.

[a] Means followed by the same letter within a trial are not significantly different at $P < 0.05$, Fisher's protected LSD.

Table 2
Mean mortality of navel orangeworm larvae infesting almonds in Block 1 after treatment with *S. carpocapsae* and *S. feltiae*

| Trial | Treatment | IJs per cm$^2$ | Percent mortality (±SE)[a] | *N* |
|---|---|---|---|---|
| November 2002 | | | | |
| | Control | | 37.3 ± 3.1 a | 212 |
| Bare plot, 500 ml | *S. carpocapsae* | 100 | 98.9 ± 4.7 b | 174 |
| Bare plot, 500 ml | *S. carpocapsae* | 10 | 96.1 ± 4.3 b | 212 |
| Bare plot, 500 ml | *S. feltiae* | 100 | 91.7 ± 4.7 b | 132 |
| Bare plot, 500 ml | *S. feltiae* | 10 | 78.9 ± 4.1 c | 225 |
| February 2003 | | | | |
| | Control | | 6.7 ± 2.7 a | 208 |
| Leaves plot, 374 ml | *S. carpocapsae* | 10 | 22.7 ± 5.6 b | 66 |
| Bare plot, 374 ml | *S. carpocapsae* | 10 | 22.8 ± 4.9 b | 92 |
| Leaves plot, 374 ml | *S. feltiae* | 10 | 34.0 ± 4.8 c | 100 |
| Bare plot, 374 ml | *S. feltiae* | 10 | 49.4 ± 5.1 d | 83 |

Infective juveniles (IJs) were applied to bare ground and leaf-covered plots. Application was followed by 374 or 500 ml water. Data from control plots were pooled.

[a] Means followed by the same letter within a trial are not significantly different at $P < 0.05$, Fisher's protected LSD.

differed in efficacy in the small plots ($P < 0.0001$, $t = 4.1$); *S. carpocapsae* caused higher mortality. In this trial, there was no difference in mortality between the two concentrations of *S. carpocapsae* but the mortality produced by *S. feltiae* was concentration dependent ($P < 0.0001$, $t = 4.8$). Overall, both species were equally effective in the large plot trial but *S. carpocapsae* caused higher mortality in half-buried almonds than in almonds on the surface ($P < 0.002$, $t = -3.2$). The efficacy of the two species reversed in Trial 2. *S. feltiae* produced greater mortality than *S. carpocapsae* ($P < 0.0001$, $t = -4.4$) and this species was more effective in bare

Table 3
Navel orangeworm larval mortality in almonds treated with 50 infective juveniles/cm$^2$ of *S. carpocapsae* and *S. feltiae* applied by an herbicide sprayer

| Treatment | Percent mortality (±SE)[a] | *N* |
|---|---|---|
| Control | 3.0 ± 2.5 a | 165 |
| *S. carpocapsae* (Nuts on surface) | 66.2 ± 4.6 b | 75 |
| *S. carpocapsae* (Nuts half buried) | 88.2 ± 6.2 c | 34 |
| *S. feltiae* (Nuts on surface) | 74.3 ± 5.8 c | 39 |
| *S. feltiae* (Nuts half buried) | 81.8 ± 10.2 c | 49 |

Data from control plots were pooled.
[a] Means followed by the same letter are not significantly different at $P < 0.05$, Fisher's protected LSD.

plots ($P < 0.009$, $t = -2.6$) while *S. carpocapsae* was equally effective in both plot types.

### 3.3. Nematode persistence in soil and multiplication in cadavers

In Trial 1, at 8 weeks after application wax moth larvae were 9× more likely to die in soil from nematode-treated plots than control plot soil ($R.R. = 9.0$, $P < 0.001$). At 12 weeks after application, only *S. feltiae* persisted ($R.R. = 3.2$, $0.025 > P > 0.01$). In Trial 4, *S. feltiae* persisted 8 weeks in Block 1 and wax moth larvae were 4.7× more likely to die in soil from *S. feltiae*-treated plots than control containers ($R.R. = 4.7$, $0.05 > P > 0.025$). The situation was reversed in Block 2 where *S. carpocapsae* persisted 8 weeks. Wax moth larvae were 1.5× more likely to die in soil from *S. carpocapsae*-treated plots than control plots ($R.R. = 1.5$, $0.025 > P > 0.01$).

NOW cadavers were examined in Trials 2 and 4 to assess the potential for nematodes to multiply in the field. In Trial 2, 23.6% ($n = 89$) of the cadavers kept at room temperature (22–24 °C) for 20 days after collection contained nematodes and in Trial 4, nematodes were observed in 51.3% ($n = 226$) of the cadavers kept at room temperature for 14 days after collection. In Trial 2 some cadavers were refrigerated after collection and very few contained nematodes (2.2%, $n = 45$).

### 3.4. Differential recovery of larvae from treated and control plots

In pistachios, there was a greater likelihood of recovering larvae (living and dead) from the control plots than the nematode-treated plots in Trials 1, 3, and 4. Larvae were 1.43×, 1.37×, and 1.32× as likely to be recovered from control than treated plots ($P < 0.001$ in each analysis). In Trial 2 the opposite occurred; larvae were 16% less likely to be recovered from control plots ($R.R. = 0.84$, $P < 0.001$). Recovery of larvae from pistachios was also dependent on time. In Trial 1 control plots, larvae were 1.85× as likely to be recovered on day 7 than day 21 ($R.R. = 1.85$, $P < 0.001$). Among the treated nuts in Trial 1, larvae were 2.3× more likely to be recovered 7 days after application than at 21 days ($R.R. = 2.3$, $P < 0.001$).

Almonds followed a similar pattern. In the small plots, differential recovery of larvae did not occur in the Trial 1 but did occur in Trial 2. In this last trial, larvae were 1.2× more likely to be recovered from control than treated almonds ($0.01 > P > 0.005$). There was no temporal difference in larval recovery in Trial 2. In the large plot experiment, larvae were 2.2× more likely to be recovered from the control plot than the nematode-treated plots ($R.R. = 2.2$, $P < 0.001$).

### 3.5. Soil temperature and relative percent saturation

In Trial 1, the daytime high temperature ranged from 15.3 to 21.4 °C and the nighttime low temperature ranged from 7.9 to 14.4 °C. In Trial 2, the daytime high temperature ranged from 22.8 to 29.4 °C and the nighttime low temperature ranged from −0.3 ° to 6.6 °C. In Trial 3, the daytime high temperature ranged from 20.3 to 26.1 °C and the nighttime low temperature ranged from 3.1 to 7.8 °C. In Trial 4, the daytime high temperature ranged from 25.3 to 30.6 °C and the nighttime low ranged from 5.6 to 10.0 °C. The difference between the highest daytime and lowest nighttime temperature in the 4 trials was 13.5, 29.7, 23.1, and 25.0 °C, respectively. The highest temperature recorded was 30.6 °C on April 27 and the lowest temperature was −0.3 °C on March 3 and 5. These soil temperatures were highly correlated with the air temperature ($P < 0.001$, data not shown).

The soil in Block 2 had a higher sand content than the soil in Block 1. The mean relative percent saturation (± SD) of the soil was 90.5 ± 6.9 for Block 1 and 32.5 ± 5.4 for Block 2 and this difference was significant ($P < 0.0001$, 8 df).

## 4. Discussion

In three trials, *S. carpocapsae* produced 15–20% greater mortality than did *S. feltiae* when applied at the same concentration. Differences in response to abiotic factors combined with differences in foraging behavior may have contributed to the greater efficacy of *S. carpocapsae*. This species is more tolerant to desiccation, hypoxia, UV, and heat than *S. feltiae* (Grewal, 2002) and as an ambush forager *S. carpocapsae* stays close to the surface (Lewis, 2002). This searching pattern may make it more effective than *S. feltiae* at locating NOW larvae inside pistachios that are on or close to the surface. Soil factors such as soil type, texture, and moisture may also have contributed to the differences observed (Barbercheck and Kaya, 1991; Koppenhöfer et al., 1995;

Kung et al., 1990). However, one could argue that the difference in efficacy noted reflects formulation differences rather than inherent differences between the two species and a different formulation of *S. feltiae* might be more effective.

In Trial 2, which was marked by the lowest mortality in the treated plots, nighttime soil temperature fell below freezing (−0.3 °C). The apparent deleterious effect of freezing temperatures of 2–4 h duration stands in contrast to Lewis and Shapiro-Ilan (2002) who reported >80% survival of *S. carpocapsae* and *S. feltiae* IJs frozen in sand at −8 °C for 1 day. Although nematode efficacy was also reduced in almonds in this trial, *S. feltiae* was more effective than *S. carpocapsae*. If these larger nuts provided more protection from cold temperatures than pistachios, and the two nematode species differed in the rate that they entered almonds, this may explain why *S. feltiae* was more effective.

We were also interested in determining whether nematode amplification occurred, as measured by increased mortality over time. Mortality did not increase over time and it is likely that most, if not all mortality, occurred during the first week after application, even though nematodes persisted for 4–8 weeks in the small plots. It is possible that amplification may have occurred in Trial 1 and been unrecognized because the high mortality at 7 days (89–95%) made it difficult to demonstrate that mortality was significantly greater at 21 days. In Trial 2, amplification may not have occurred because the temperature dropped below the developmental threshold for *S. carpocapsae*. Lewis and Shapiro-Ilan (2002) reported that nematodes are most susceptible to freezing 48–72 h post infection, and freezing may have occurred when the nematodes were most vulnerable. In order to address the question of nematode amplification in the orchard, a combination of laboratory and field studies is necessary.

Differential recovery of larvae between treated and control plots, as well as decreased recovery over time, was noted in several trials and may have occurred for the following reasons. First, exposure to nematodes may have made larvae more likely to leave the nut. In our laboratory, third and fourth instar NOW larvae in petri dishes exposed to >100 IJs/cm$^2$ were more active during a 6 h period than larvae that were unexposed. Second, if dead larvae were more likely to be consumed or removed by scavengers, fewer larvae would be recovered from nematode-treated plots. Baur et al. (1998) reported that steinernematid-killed *Galleria mellonella* cadavers were more likely to be scavenged by ants than heterorhabditid-killed cadavers. Conversely, Zhou et al. (2002) reported that *Xenorhabdus nematophila* (Thomas and Poinar), the mutualistic bacterium associated with *S. carpocapsae*, produced factors that were repellent to ants. Removal of cadavers by ants was not specifically addressed in our study but we also noted several Dipteran families (Muscidae, Sarcophagidae, and Tipulidae) inside nuts and they may have consumed cadavers. Third and finally, it is more difficult to find small cadavers as well as larvae consumed by nematodes in rotten nuts, and the greater number of live larvae recovered may reflect the limit of our searching ability. These hypotheses are not mutually exclusive and mortality was probably underestimated in this study.

Our goal was to replicate field conditions as closely as possible. Coverage of nuts with varying amounts of soil is representative because in the field, some nuts lie on other nuts at the surface while others are almost completely buried. The nuts are not static and are moved by rain, which can also deposit soil on pistachios. Pistachio leaves fall on the berm and between rows in mid to late November and the leaf-covered plots replicated the depth that these leaves cover the berm. Our use of screens in this study was a necessary evil because we needed to protect nuts from birds and foraging mammals (fewer pistachios were recovered when they were not used). Although screens may have altered the microenvironment, the results from Trial 3 indicate that *S. carpocapsae* was still effective when they were not used and we conclude that screens were not a source of bias.

Almonds were included in the study for several reasons. First, inclusion of almonds allowed comparison of differences in NOW mortality between artificially and naturally infested nuts. We conclude that artificial infestation did not increase the susceptibility of NOW to nematodes because there was no difference in NOW mortality between almonds and pistachios. Second, we wanted to compare our findings to previous studies, which were all conducted on almonds. The mortality in our study was comparable to Lindegren et al. (1987) who reported 78% mortality in artificially infested almonds in trees and greater than the 11.8% larval mortality reported by Agudelo-Silva et al. (1995) in infested almond mummies. We believe that our study achieved greater control using a lower concentration of nematodes because ground application is compatible with the biology of both nematode species. Finally, the use of almonds as sentinels enabled us to evaluate the effectiveness of a large scale application because the availability of infested pistachios was a limiting factor.

In summary, *S. carpocapsae* consistently and effectively controlled NOW in small plots and was effective when applied over pistachio leaves. This last point is important because late fall or winter is the time when most of the nuts are on the ground. The rate of 3740 liters/ha used in this study is the upper limit of what is practical for the field and this technology is much more likely to be adopted if lower application rates can be used. Currently, *S. carpocapsae* costs ≈$75 per billion nematodes and at the concentration of 10 IJs/cm$^2$ the cost per ha is at the threshold of affordability. Future

small plot studies will assess the effect of other combinations of nematode concentrations and lower application rates on NOW mortality.

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